

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-12252- -2	FECHA: 2015-08-24 19:11:13
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 379 FASE NACIONAL INCLUIDO CAPITULO II
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 4
ACT: 430 REQUERSOLICITA	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JUAN CARLOS CUESTA QUINTERO

Asunto: Radicación: 14-12252- -2
Trámite: 11
Evento: 379
Actuación: 430
Oficio: 10126
Folios: 4

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I		Capítulo II	X
No. Solicitud Internacional	PCT/US2012/048543				
Fecha de Presentación Internacional	27/07/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N°14-12252-00000-0000	22/Enero/2014
Reivindicaciones:	Radicado N°14-12252-00000-0000	22/Enero/2014
Dibujos:	Radicado N° 14-12252-00000-0000	22/Enero/2014
Secuencias:	Radicado N° 14-12252-00000-0000	22/Enero/2014
Prioridad:	US 61 513 259	29/Julio/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las

reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 12 del capítulo reivindicatorio (Radicado N°. 14-12252-00000-0000).

Título de la solicitud: “RECEPTORES COESTIMULADORES DE CAMBIO”

El problema planteado en esta solicitud consiste en la necesidad de obtener nuevas proteínas de fusión quimérica.

Para resolver este problema técnico la solicitud en estudio proporciona una nueva proteína de fusión quimérica de manera que cuando se manifiesta en una célula puede convertir en una señal negativa en una señal positiva en la célula.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): AG1K 48/00; A61K 38/17.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 10-12 no es patentable de acuerdo con el Art citado, porque menciona la administración Para tratar un sujeto que lo necesita, por lo tanto es considerado una excepción a la patentabilidad

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1-6 no son claras puesto que mencionan una proteína de fusión, la cual no se encuentra caracterizada estructuralmente, lo cual no permite distinguir el objeto de la invención del estado de la técnica. Se sugiere a la solicitante mencionar las características estructurales de la proteína de fusión, es decir, por medio de las SEQ ID No que conforman dicha proteína de fusión.

Las reivindicaciones 1-2, 4-9 no son claras puesto que mencionan “*donde dicho primer dominio es un polipéptido que está asociado con una señal negativa y dicho segundo dominio es un polipéptido que está asociado con una señal positiva*”, “*un dominio de reconocimiento de antígeno de un anticuerpo específico y un dominio intracelular de la cadena CD3-zeta*”, lo cual es considerado un resultado a alcanzar y no define la proteína de fusión por sus características estructurales, puesto que las reivindicaciones incluirían no sólo la solución propuesta por la solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, que contribuyen a la obtención de ese efecto.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 29 de julio de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	Dennehy KM1, Elias F, J Immunol.	Cutting edge: monovalency of CD28 maintains the antigen dependence of T cell costimulatory responses.	17/Mayo/2006

El documento D1¹ divulga construcciones bivalentes de CD28 mediante la fusión de los dominios extracelulares de CTLA-4 o CD80 con los dominios intracelulares de CD28 (Resumen).

7. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en: *“Una proteína de fusión que comprende un primer dominio y un segundo dominio, en donde dicho primer dominio es un polipéptido que está asociado con una señal negativa y dicho segundo dominio es un polipéptido que está asociado con una señal positiva.”* (Radicado N°. 14-12252-00000-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Una proteína de fusión que comprende un primer dominio y un segundo dominio, en donde dicho primer dominio es un polipéptido y dicho segundo dominio es un polipéptido	Una proteína de fusión que comprende un primer dominio y un segundo dominio, en donde dicho primer dominio es un polipéptido y dicho segundo dominio es un polipéptido (Pág. 5726, Col. izq., Fig. 1)

Las características esenciales de la proteína de fusión de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual da a conocer una proteína de fusión que comprende un primer dominio y un segundo dominio, donde las construcciones son bivalentes de CD28 mediante la fusión de los dominios extracelulares de CTLA-4 o CD80 con los dominios intracelulares de CD28 (Pág. 5726, Col. izq., Fig. 1).

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2-9 no mencionan alguna característica que haga nuevo la proteína de fusión de la reivindicación 1, por lo cual se considera que no son nuevas.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Dennehy KM1, Elias F, J Immunol.	1-9	Novedad

¹ <http://www.jimmunol.org/content/176/10/5725.full.pdf+html>

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-09-01

Elaboró: Juanita Andrea Carvajal
Revisó: Sandra Carolina Crespo

INFORME DE BÚSQUEDA DE PATENTES PARA EL No de Radicación: 14-12252-00000-0000						
CRITERIOS PARA LA BÚSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)						
Palabras clave utilizadas		Proteína de fusion, CD28, CTLA4.				
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA		AG1K 48/00, A61K 38/17				
BASES DE DATOS CONSULTADAS.						
Patentscope		jimmunol				
Google/patents						
jimmunol						
INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS (documentos patentes, literatura no patente etc...)						
Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
EUROPEA	16/10/2012	Dennehy, Cutting Edge: Monovalency of CD28 Maintains the Antigen Dependence of the T Cell Costimulatory Responses	1-12	Novedad/Nivel Inventivo	x	
		Zhao, Multiple Injections of Electroporated Autologous T Cells Expressing a Chimeric Antigen Receptor Mediate Regression of Human Disseminated Tumor. Cancer	8,11,12	Nivel Inventivo		x
		Carpenito, Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains	10-12	Nivel Inventivo		x
		Paulos, Putting the brakes on BTLA in T cell-mediated cancer immunotherapy.	10-12	Nivel Inventivo		x
		US 2010/0316646	1-12	Nivel Inventivo		x
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
BÚSQUEDA DE SECUENCIAS						
Número de depósito / patente y N° de secuencia % de identidad						
SEQ ID NO (de la solicitud)	NCBI	The Lens	EMBL-EBI	DDJB	Genome Quest	
N.A						



PROTEIN OF fusion CD28



Académico

Aproximadamente 32.800 resultados (0,09 s)

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Sugerencia: Buscar solo resultados en **español**. Puedes especificar el idioma de búsqueda en Configuración de Google Académico.

B70 antigen is a second ligand for CTLA-4 and CD28

M Azuma, D Ito, H Yagita, K Okumura, JH Phillips... - Nature, 1993 - nature.com

... **fusion protein** to human B-lymphoblastoid cell lines and, together with anti-B7 antibody, completely blocked CTLA-4 binding. Further IT2 efficiently inhibited primary allogeneic mixed lymphocyte responses. These findings indicate that B70 is a second ligand for **CD28** and CTLA-4 ...

Citado por 863 Artículos relacionados Las 9 versiones Citar Guardar

Mechanism for down-regulation of CD28 by Nef

nih.gov [HTML]

T Swigut, N Shohdy, J Skowronski - The EMBO journal, 2001 - emboj.embopress.org

... Abstract. SIV and HIV Nef **proteins** disrupt T-cell receptor machinery by down-modulating cell surface expression of CD4 and expression or signaling of CD3-TCR. ... In cells expressing a Nef-green fluorescent **protein** (GFP) **fusion**, **CD28** co-localizes with the AP-2 clathrin ...

Citado por 210 Artículos relacionados Las 11 versiones Citar Guardar

Negative-feedback regulation of CD28 costimulation by a novel mitogen-activated protein kinase phosphatase, MKP6

jimmunol.org [HTML]

F Marti, A Krause, NH Post, C Lyddane... - The Journal of ... 2001 - Am Assoc Immunol

... complete medium for 36 h. Cells were lysed in Nonidet P-40 lysis buffer, and 1 ml of lysate containing 0.5 mg of **protein** was incubated with 10 µl of packed glutathione-agarose beads coated with 4 µg of GST-**CD28**, CD28Δ199, or p58 KIR clone 6 CYT **fusion proteins** for 30 min. ...

Citado por 52 Artículos relacionados Las 4 versiones Citar Guardar

Covalent dimerization of CD28/CTLA-4 and oligomerization of CD80/CD86 regulate T cell costimulatory interactions

jbcb.org [HTML]

JAL Greene, GM Leytze, J Emswiler, R Peach... - Journal of Biological ... 1996 - ASBMB

... Polymerase chain reaction products encoding the extracellular domains of **CD28** and CTLA4lg (9 ... Ig **fusion proteins** were produced by transiently transfected COS cells or stably transfected ... ovary cells and purified by affinity chromatography on immobilized **protein** A-Sepharose ...

Citado por 172 Artículos relacionados Las 4 versiones Citar Guardar

Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation

rupress.org [PDF]

PS Linsley, W Brady, L Grosmaire, A Aruffo... - The Journal of ... 1991 - jem.rupress.org

... the functional activities of B7 Ig and **CD28** Ig, we first tested binding to CHO cell lines expressing **CD28** or B7. In early experiments, spent culture media from transfected COS cells was used as a source of **fusion protein**, while in later experiments, purified **proteins** were used (Fig ...

Citado por 1164 Artículos relacionados Las 13 versiones Citar Guardar

Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4lg

researchgate.net [PDF]

JM Kremer, R Westhovens, M Leon... - England Journal of ... 2003 - Mass Medical Soc

... A **fusion protein** — cytotoxic T-lymphocyte-associated antigen 4-IgG1 (CTLA4lg) — is the first in a new class of drugs known as costimulation ... CTLA4lg binds to CD80 and CD86 on antigen-presenting cells, blocking the engagement of **CD28** on T cells and preventing T-cell ...

Citado por 581 Artículos relacionados Las 11 versiones Citar Guardar

CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation

rupress.org [PDF]

MF Krummel, JP Allison - The journal of experimental medicine, 1995 - jem.rupress.org

... This higher affinity probably accounts for the ability of the CTLA-4 Ig **fusion protein** to block costimulation in vitro (10, 12) and to suppress graft rejection and antibody production in vivo (19, 20). While the ability of CTLA-4 **fusion proteins** to bind **CD28** ligands and block T cell ...

Citado por 1055 Artículos relacionados Las 11 versiones Citar Guardar

Adenovirus-mediated gene transfer of CTLA-4lg fusion protein in the suppression of experimental autoimmune arthritis

E Quattrocchi, MJ Dallman... - Arthritis & Rheumatism, 2000 - Wiley Online Library

... Blockade of **CD28**-B7 interactions with soluble CTLA-4lg **fusion protein** (which binds ... inhibition of this costimulatory pathway may be required, and the adenovirus-mediated gene-transfer technology is very efficient in achieving sustained expression of **proteins** in vivo ...

Citado por 65 Artículos relacionados Las 4 versiones Citar Guardar

Growth factor receptor-bound protein 2 SH2/SH3 domain binding to CD28 and its role in co-signaling

jbcb.org [HTML]

HH Kim, M Tharayil, CE Rudd - Journal of Biological Chemistry, 1998 - ASBMB

... Analysis of **CD28** binding by SH2 and SH3 domains of Grb2. A and B, **CD28 protein** co-expressed with Lck by the baculovirus system was precipitated with GST-Grb2 **fusion proteins** (1 µg) or anti-**CD28** (4B10) plus rabbit anti-mouse. ...

Citado por 120 Artículos relacionados Las 5 versiones Citar Guardar

CD3/CD28 costimulation-induced NF-κB activation is mediated by recruitment of protein kinase C-θ, Bcl10, and IκB kinase β to the immunological synapse through ...

asm.org [HTML]

D Wang, R Matsumoto, Y You, T Che... - ... and cellular biology, 2004 - Am Soc Microbiol

... CARMA1, a MAGUK domain-containing **protein**, plays an essential role in CD3 ... MAGUK domain-containing **proteins** are generally involved in organization of multiprotein complexes ... κB activation, whereas expression of a Bcl10-CARMA1(ΔCD) **fusion protein** effectively rescues ...

Citado por 195 Artículos relacionados Las 11 versiones Citar Guardar

PROTEIN OF fusion CD28 AND CLA4



Web Imágenes Videos Noticias Más Herramientas de búsqueda

Cualquier país - Cualquier idioma - Antes del 29 Jul, 2011 - Ordenar por releva

Artículos académicos para PROTEIN OF fusion CD28 AND CLA4

CD59, an LY-6-like protein expressed in human ... - Davies - Mencionado por 539
... QSR1: two Ste20-related protein kinases regulating ion ... - Gagnon - Mencionado por 36
Polypeptide fragments of human PAK5 protein kinase - Plowman - Mencionado por 8

Mechanism for down-regulation of CD28 by Nef

www.ncbi.nlm.nih.gov ... PubMed Central (PMC) Traducir esta página
por T. Svigut - 2001 - Mencionado por 210 - Artículos relacionados
23 ago. 2003 - In cells expressing a Nef-green fluorescent protein (GFP) fusion, CD28 co-localizes with the AP-2 clathrin adaptor and Nef-GFP. Mutations that disrupt Nef ...

B70 antigen is a second ligand for CTLA-4 and CD28.

www.ncbi.nlm.nih.gov/pubmed/7654153 Traducir esta página
por M. Azuma - 1993 - Mencionado por 864 - Artículos relacionados
4 nov. 1993 - Interaction between CD28 and B7 results in potent costimulation of T-cell ... a CTLA4-immunoglobulin fusion protein to human B-lymphoblastoid cell lines and ...

The Cdc42 Effectors Ste20, Cla4, and Skm1 Down-Regulate ...

www.ncbi.nlm.nih.gov ... PubMed Central (PMC) Traducir esta página
por M. Lin - 2009 - Mencionado por 14 - Artículos relacionados
7 oct. 2009 - Ste20 and Cla4 also bind to Bem1, a scaffold protein that brings Cdc42, ... However, the GFP-NLS fusion protein was found in the cytoplasm (data not shown).
Falta(n): ed26

Refolding and characterization of recombinant human GST ...

www.ncbi.nlm.nih.gov/pubmed/14970911 Traducir esta página
por DW Li - 2004 - Mencionado por 8 - Artículos relacionados
15 feb. 2004 - Programmed death-1 (PD-1) is a costimulatory molecule of CD28 family ... The fusion protein GST-PD-1 was effectively expressed in E. coli BL21 (DE3) as ...

A D- A 273 493 nO eompoleting

www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA273493 Traducir esta página
por T. Lindsten - 1993 - Mencionado por 283 - Artículos relacionados
3 ago. 2008 - recta the constitutive expression of fusion proteins in which cytotoxic T ... epitiated by CD28 mAb 9.3 under reducing conditions from similarly in CLA-4 (17)

New Treatments for Lupus by Daniel J. Wallace, MD

es.slideshare.net/.../new-treatments-for-lupus-by-dan... Traducir esta página
2 jul. 2008 - <ub><lo>CD80/86 CD28 is one of the best characterized co-stimulatory pathways ... activator) Renal cancer Leukemia Anti-CD28 (TGN1412) Tumors Anti-CLA-4 ... APRIL <ub><s>Atacicept is a fusion protein formed between the extracellular ...

Management of Prostate Cancer - Mayo Medical Laboratories

www.mayomedicallaboratories.com/articles/.../03.htm Traducir esta página
5 mar. 2010 - Fusion of TMPRSS2 with ERG results in androgen regulation of ERG and is detected in ... Ligand activation triggers dissociation of the AR-heat shock protein complex, ... with CD28) binds to the same ligands present on antigen-presenting cells: ... allogenic whole cell-based, Atrasentan Sipuleucel-T and GVAX; anti-CLA4 ...

cd59, an ly6-like protein expressed in human lymphoid cells ...

jem.rupress.org/content/170/3/637.full.pdf Traducir esta página
por A. Davies - 1989 - Mencionado por 539 - Artículos relacionados
4 nov. 2004 - protein interferes with the assembly of the C3 converting enzymes both of the clas-sical and ... CAMPATH-2) were obtained from the fusion of a DA rat spleen with the myeloma line ... Negative: CLA4 Negative: HALM-1 Negative: Negative: NALM-6. Negative ... Molecular cloning of a CD28 cDNA by a high efficiency ...

C-C Chemokine Receptor 4 Expression Defines a Major ...

www.jimmunol.org/content/166/1/103.full Traducir esta página
por DP Andrew - 2001 - Mencionado por 186 - Artículos relacionados
1 ene. 2001 - The mononuclear cells were allowed to adhere to protein-coated plastic for 2-3 h, ... six-well Falcon plates were coated overnight with 10 µg/ml anti-CD28 and 2 µg/ml ... Generally, 3000-5000 hybridomas were generated per fusion and the ...

Proteína de fusión, CD28, CTLA4.



Web

Imágenes

Noticias

Videos

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Herramientas de búsqueda

Cualquier país ▾

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Antes del 29 jul. 2011 ▾

Ordenar por rel

CTLA-4 - iqb

www.iqb.es/reumatologia/fichas/ctla.html ▾

22 may. 2009 - Sin embargo, mientras que la CD28 lanza una señal estimulante, la CTLA-4 envía ... Se han utilizado proteínas de fusión de la CTLA-4 con anticuerpos en el ...

moléculas accesorias, cd28, revista de inmunología ...

encolombia.com ▾ ... ▾ Revista de Inmunología ▾ Volumen 09 nº 4 ▾

1 feb. 2002 - El CD28 humano presenta extensa homología al CD28 de otras especies de ... IL-4 al bloquear las interacciones CD28/B7 con la proteína de fusión hCTLA4-Ig (16). ... El antígeno asociado al linfocito T citolítico (CTLA-4), deriva su nombre de ...

Versión HTML - Acta Médica Colombiana

www.actamedicacolombiana.com/anexo/articulos/02-2002-08.htm ▾

1 sept. 2002 - Palabras clave: moléculas coestimuladoras, inmunomodulación, CD28, CD 152, Es así como al utilizar la molécula CTLA-4 como proteína de fusión que ...

Co-receptor negativo en linfocitos T. Interacción CTLA-4 y ...

www.portalesmedicos.com/.../Co-receptor-negativo-en-linfocitos-T-Inter... ▾

por AY González - Artículos relacionados

13 ago. 2010 - Algunas de estas proteínas son moléculas de adhesión y otras dan lugar a ... IL-4 al bloquear las interacciones CD28/B7 con la proteína de fusión CTLA4 Ig. De ...

[PDF] Eficacia de abatacept en la artritis reumatoide. Actualización

files.sld.cu/reuma/.../eficacia-de-abatacept-en-la-artritis-reumatoide.pdf ▾

1 jul. 2010 - CTLA4, que tiene efectos contrarios al. CD28, se expresa también en la superficie del linfocito T3,4. El abatacept, es una proteína de fusión recombinante ...

[PDF] Descargar - Telmeds.org

www.telmeds.org/wp-content/.../Resumen-del-capitulo-9-del-Abbas1.pdf ▾

23 jun. 2011 - unen a proteínas propias y las modifican, creando nuevos determinantes CD28. Las señales mediadas por CD28 aumentan la producción de citosinas ... CTLA-4Ig, una proteína de fusión formada por el dominio extracelular de CTLA-4 y la ...

La CTLA4-IG, una promesa en la I+D de fármacos proteínicos

www.diariomedico.com/.../ctla4-ig-una-promesa-en-id-farmacos-proteini... ▾

2 sept. 2009 - Las proteínas terapéuticas o los fármacos proteínicos ganan terreno. Un ejemplo de alta innovación es el desarrollo de una nueva proteína de fusión cuya ...

Revista médica de Chile - La inmunología clínica actual ...

www.scielo.cl/scielo.php?pid=S0034-98872000000600013&script... ▾

por F Carrión - 2000 - Mencionado por 2 - Artículos relacionados

15 jun. 2000 - Proteína relacionada con la organización del citoesqueleto. Los estudios funcionales demostraron que el CD28 y el CTLA4 son muy importantes ... vivo con una proteína de fusión construida a partir del dominio extracelular del CTLA4 y la ...

Nuevas dianas terapéuticas en artritis reumatoide ...

www.reumatologiainmunologia.org/es/nuevas-dianas...artritis.../13092262/ ▾

2 sept. 2006 - ... una proteína de fusión CTLA4 (abatacept), lo que ha reactivado el interés por la célula T como diana celular, aunque es posible que las funciones del CD28 ...

Inducción de tolerancia en el trasplante de órganos sólidos ...

www.elsevier.es ▾ Inicio ▾ Gastroenterología y Hepatología ▾

15 oct. 2004 - ... CD4⁺CD25⁺, Tr1 y una población de células¹⁹ T CD8β⁺CD28CD27⁺. que la administración de la proteína de fusión CTLA4-Ig, que impide que CD28 se ...

PROTEIN OF fusion CD28 AND CLA4



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Cerca de 288 resultados (0.44 segundos)

Pharmaceutical compositions comprising immunologically ...

www.google.com/patents/EP2316479B1?cl... - Traducir esta página
Otorgada - Presentada el 18 Jul. 2003 - Emitido el 3 Dic. 2014 -
Lawrence Corey - University of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and sorted into two ... In one embodiment, the fusion protein is soluble.
Descripción general - Información relacionada - Debate

Lentiviral vectors and uses thereof

www.google.com/patents/US8828727 - Traducir esta página
Otorgada - Presentada el 23 Mayo 2005 - Emitido el 9 Sep. 2014 -
Wayne A. Marasco - Dana-Farber Cancer Institute
The fusion protein contains a leader peptide, a candidate polypeptide, ...
For example, transmembrane moiety is the lentiviral gp41, CD28, CD8 or IgM ... at the 5' end of the gag gene bounded by a Cla I site (see, e.g., FIG.
Descripción general - Información relacionada - Debate

Pharmaceutical compositions comprising immunologically ...

www.google.com/patents/EP2011510B1?cl... - Traducir esta página
Otorgada - Presentada el 18 Jul. 2003 - Emitido el 12 Ene. 2011 -
Lawrence Corey - University of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and sorted into two ... In one embodiment, the fusion protein is soluble.
Descripción general - Información relacionada - Debate

Pharmaceutical compositions comprising immunologically ...

www.google.com/patents/EP2263686B1?cl... - Traducir esta página
Otorgada - Presentada el 18 Jul. 2003 - Emitido el 26 Dic. 2012 - David
M. Koelle - University of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and sorted into two ... In one embodiment, the fusion protein is soluble.
Descripción general - Información relacionada - Debate

Modified sFv molecules which mediate adhesion between ...

www.google.com/patents/US6699715 - Traducir esta página
Otorgada - Presentada el 27 Nov. 1996 - Emitido el 2 Mar. 2004 - Jeffrey
A. Ledbetter - Bristol-Myers Squibb Co.
The signal to the T cells is specific for the CD28 receptor, and that the ... at
0.5 µg/ml or 9.3/L5 sFvlg (—) fusion protein at 0.5 µg/ml or with ...
from clone 2V57#5 was ligated into the Hind-III/Cla-I cloning sites of
pLNCX.
Descripción general - Información relacionada - Debate

Rapid, efficient purification of hsv-specific ...

www.google.com/patents/EP1523582B1?cl... - Traducir esta página
Otorgada - Presentada el 18 Jul. 2003 - Emitido el 12 Nov. 2008 - David
M. Koelle - University of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and sorted into two ... In one embodiment, the fusion protein is soluble.
Descripción general - Información relacionada - Debate

Rapid, efficient purification of hsv-specific ...

www.google.com/patents/WO2004009821 - Traducir esta página
Solicitud - Presentada el 18 Jul. 2003 - Publicada el 29 Ene. 2004 -
David M Koelle - Univ Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and sorted into two ... In one embodiment, the fusion protein is soluble.
Descripción general - Información relacionada - Debate

Rapid, efficient purification of hsv-specific ...

www.google.com/patents/US20130189294 - Traducir esta página
Solicitud - Presentada el 15 Mar. 2013 - Publicada el 25 Jul. 2013 -
David M. Koelle - University Of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and ... A
soluble fusion protein of the invention can be suitable for injection ...
Descripción general - Información relacionada - Debate

Rapid, efficient purification of hsv-specific ...

www.google.com/patents/US20130011425 - Traducir esta página
Solicitud - Presentada el 14 Mayo 2012 - Publicada el 10 Ene. 2013 -
David M. Koelle - University Of Washington
PBMC are stained with mAb specific for CD6a, CD28, and CLA, and ... A
soluble fusion protein of the invention can be suitable for injection ...
Descripción general - Información relacionada - Debate

Rapid, efficient purification of HSV-specific ...

www.google.com/patents/US7431934 - Traducir esta página
Otorgada - Presentada el 28 Feb. 2006 - Emitido el 7 Oct. 2008 - David

Methods and compositions for modulating T cell and/or B ...



www.google.com/patents/US8501677 - Traducir esta página
Otorgada - Presentada el 28 Oct. 2011 - Emitido el 6 Ago. 2013 - Jenny
P.-Y. Ting - University Of North Carolina At Chapel Hill
The method of claim 1, wherein the fusion protein comprising the ... pairs
on T cells and DCs are CD154-CD40 and CD28-B7 (T-DC respectively). ...
Tysabri (anti-VLA4) and anti-CLA4 Ab. Other inhibitors that can be used
in ...
Descripción general - Información relacionada - Debate

Compositions and methods to regulate an immune response ...



www.google.com/patents/US20030219436 - Traducir esta página
Solicitud - Presentada el 15 Mar. 2002 - Publicada el 27 Nov. 2003 -
Jeffrey Ledbetter - Ledbetter Jeffrey A.
Without a second signal via CD28, ... in another immobilized form, CD63-
Ig fusion protein is bound to a solid surface. ... cgt ctc c (SEQ ID NO:1)
and a 3' antisense primer that includes a Cla I site: cctagcta tca acc agc tct
gtc ...
Descripción general - Información relacionada - Debate

Uses of monoclonal antibody 8H9



www.google.com/patents/US8501471 - Traducir esta página
Otorgada - Presentada el 9 Jun. 2010 - Emitido el 6 Ago. 2013 - Nai-
Kong V. Cheung - Sloan-Kettering Institute For Cancer Research
This invention provides a protein with about 58 kilodaltons in molecular
... of TCR functions. Furthermore scFv-fusion proteins (e.g. CD28,
... hCD8a-leader-scFv3G6-CD28) cassette of pMSCVneo using the Cla I-
NotI ...
Descripción general - Información relacionada - Debate

Methods of preparing and using single chain anti-tumor ...



www.google.com/patents/US7666424 - Traducir esta página
Otorgada - Presentada el 17 Oct. 2002 - Emitido el 23 Feb. 2010 - Nai-
Kong Cheung - Sloan-Kettering Institute For Cancer Research
Expression of such fusion proteins in T cells leads to presentation of ...
hCD8a-leader-scFv3G6-CD28) cassette of pMSCVneo using the Cla ...
Descripción general - Información relacionada - Debate

Inhibitors of vascular endothelial growth factor activity, ...



www.google.com/patents/US6383486 - Traducir esta página
Otorgada - Presentada el 1 Jul. 1999 - Emitido el 7 Mayo 2002 - Teri
Lynn Davis-Smyth - Genentech, Inc.
"Flk2" is the chimeric protein having only flk-1 ig-like domain 2 fused to
the Fc of IgG ... 61:1303-1313 [1990], CD28 and B7 (Linsley et al., ...
using PCR primers containing restriction sites (Cla I and Bst BI, 5' and
3' ...
Descripción general - Información relacionada - Debate

Inhibitors of vascular endothelial growth factor activity, ...



www.google.com/patents/US6897294 - Traducir esta página
Otorgada - Presentada el 20 Mar. 2002 - Emitido el 24 Mayo 2005 - Teri
Lynn Davis-Smyth - Genentech, Inc.
"Flk2" is the chimeric protein having only flk-1 ig-like domain 2 fused to
the ... 61:1303-1313 [1990], CD28 and B7 (Linsley et al., ... Cla I)
and ...
Descripción general - Información relacionada - Debate

Influenza vaccines containing modified adenovirus vectors



www.google.com/patents/US20140377295 - Traducir esta página
Solicitud - Presentada el 23 Mayo 2012 - Publicada el 25 Dic. 2014 -
Hildegund C. Ertl - The Wistar Institute Of Anatomy And Biology
The fusion protein of claim 8, further comprising a nucleoprotein ... for
6 hrs with anti-CD28 (clone CD28.2), anti-CD49d (clone 9F10), and
Briefeldin A. 14, ... 5' oligonucleotides containing the Cla I site of hexon
followed by ...
Descripción general - Información relacionada - Debate

Polypeptides involved in immune response



www.google.com/patents/US7708993 - Traducir esta página
Otorgada - Presentada el 21 Feb. 2006 - Emitido el 4 Mayo 2010 -
Steven K. Yoshinaga - Amgen Inc.
The polypeptides are referred to as CD28 related protein-1, or CRP1, ...
mCRP1-Fc, mB7RP1, and mB7.2-Fc fusion proteins were added to ...
The purified plasmid DNA was digested with the restriction enzymes, Cla I
and ...
Descripción general - Información relacionada - Debate

Proteins that bind p16 and uses thereof



www.google.com/patents/WO2011127543 - Traducir esta página
Solicitud - Presentada el 18 Abr. 2011 - Publicada el 20 Oct. 2011 -
Simon Barry - Transbio Ltd
The present disclosure provides an isolated protein comprising at least ...
and one or more proteins selected from the group consisting of CLA,
HLA-DP and CCR4 ... P16 fused to a Fc and assessing binding of the
protein (e.g., a Fab) ... in the presence of recombinant human TGFP-1
...
Descripción general - Información relacionada - Debate



Cutting Edge: Monovalency of CD28 Maintains the Antigen Dependence of T Cell Costimulatory Responses

This information is current as of August 24, 2015.

Kevin M. Dennehy, Fernando Elias, Gabrielle Zeder-Lutz, Xin Ding, Danièle Altschuh, Fred Lühder and Thomas Hünig

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doi: 10.4049/jimmunol.176.10.5725
<http://www.jimmunol.org/content/176/10/5725>

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http://www.jimmunol.org/content/176/10/5725.full#ref-list-1 |
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CUTTING EDGE

Cutting Edge: Monovalency of CD28 Maintains the Antigen Dependence of T Cell Costimulatory Responses¹

Kevin M. Dennehy,^{*†} Fernando Elias,^{*} Gabrielle Zeder-Lutz,[‡] Xin Ding,[§] Danièle Altschuh,[‡] Fred Lühder,^{*¶} and Thomas Hünig^{2*}

CD28 and CTLA-4 are the major costimulatory receptors on naive T cells. But it is not clear why CD28 is monovalent whereas CTLA-4 is bivalent for their shared ligands CD80/86. We generated bivalent CD28 constructs by fusing the extracellular domains of CTLA-4 or CD80 with the intracellular domains of CD28. Bivalent or monovalent CD28 constructs were ligated with recombinant ligands with or without TCR coligation. Monovalent CD28 ligation did not induce responses unless the TCR was coligated. By contrast, bivalent CD28 ligation induced responses in the absence of TCR engagement. To extend these findings to primary cells, we used novel superagonistic and conventional CD28 Abs. Superagonistic Ab D665, but not conventional Ab E18, predominantly ligates CD28 bivalently at low CD28/Ab ratios and induces Ag-independent T cell proliferation. Monovalency of CD28 for its natural ligands is thus essential to provide costimulation without inducing responses in the absence of TCR engagement. The Journal of Immunology, 2006, 176: 5725–5729.

The destructive potential of the immune system necessitates tight control of effector mechanisms by positive and negative regulatory receptors. CD28 and CTLA-4 comprise one such pair of costimulatory receptors controlling proliferation and differentiation of naive T cells (1, 2).

Almost all physiological responses of naive T cells require costimulation via engagement of the clonotypic TCR by the appropriate Ag/MHC and by CD28 and its ligands CD80 and CD86 (3). Under exceptional circumstances, triggering of the TCR/CD3 complex alone is sufficient to induce proliferation. Examples include stimulation with CD3-specific Abs at high surface density (4) or physiological responses to high avidity Ag from the noncytolytic lymphocytic choriomeningitis virus (3, 5). At the other extreme of costimulatory responses, CD28-specific Abs referred to as “superagonists” are able to induce pro-

liferation without TCR engagement (6, 7). But the mechanism of superagonist Ab stimulation remains obscure, and there is no physiological equivalent of such T cell activation.

Although CD28 and CTLA-4 are both homodimers, they bind to their shared ligand, CD80, differently (Fig. 1A). The cocrystal structure of CTLA-4 and CD80 shows that the two Ig domains of CTLA-4 form a V structure in which each arm is accessible to bind separate CD80 molecules (8). Because CD80 is predominantly also a bivalent homodimer, CTLA-4 and CD80 form an unusually stable lattice structure. Biacore studies demonstrate that CD28, in contrast to CTLA-4, is functionally monovalent (9). To date there is no crystal structure of the CD28 homodimer. However, the structure of the human CD28 monomer in complex with a superagonist CD28 Ab Fab' has recently been solved (10). Modeling based on this structure suggests that the CD28 Ig domains are arranged in a U structure. Although both arms are available for ligand binding, simultaneous binding of separate CD80 monomers would be prevented by a physical clash of the C-set domains. The CD28 homodimer is thus functionally monovalent, whereas CTLA-4 is bivalent.

In this study, we have addressed the influence of valency on CD28 function using CD28 chimeric molecules and superagonistic CD28-specific Abs. We show that monovalency of CD28 is essential for physiological costimulation. Monovalent ligation of CD28 induces efficient costimulation without inducing responses in the absence of TCR ligation, whereas bivalent ligation with natural ligands alone induces T cell activation. These results indicate that monovalency of CD28/ligand interactions is an important safeguard that keeps T cell activation under the control of the TCR under physiological conditions.

Materials and Methods

Abs, cell lines, and constructs

Abs to rat TCR $\alpha\beta$ (R73), and recombinant rat CTLA-4IgGFc have been described previously (11, 12). Recombinant mouse CD28IgGFc and human CD80IgGFc were from R&D Systems, sheep anti-mouse IgG was from Roche, and mouse anti-human IgG was from Dianova.

Chimeric CD28, comprising the extracellular region of rat CD28 (rCD28)³ and the intracellular regions of mouse CD28 (mCD28), and chimeric mCD28,

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² Address correspondence and reprint requests to Prof. Thomas Hünig, Institute for Virology and Immunobiology, University of Würzburg, Versbacherstrasse 7, 97078 Würzburg, Germany. E-mail address: huenig@vim.uni-wuerzburg.de

³ Abbreviations used in this paper: rCD28, rat CD28; mCD28, mouse CD28; RU, resonance unit(s).

grafted with the human C'D loop (aa 60–65) and the rat ligand binding region (m/rCD28 1–66), have been described (7). To generate the CTLA-4mCD28 chimera, mCD28 intracellular domains amplified using 5'-TTTGGATCCTG GTCGTGGTTGCTGGAGT-3' AND 5'-ATATAAGATCTTCAGGGGCG GGTACGCTGCAAA-3' were digested with *Bam*H1/*Bgl*11. The human CTLA-4 extracellular domain was amplified using 5'-CAGAATTCCACCAT GGCTTGCCTTGGATTTCAGCG-3' and 5'-CAAGGATCCAGAGGAG GAAGTCAGAACTCTGGGCACG-3', digested with *Eco*R1/*Bam*H1, and ligated with the mCD28 fragment into pczCFG5IEGN *Eco*R1/*Bam*H1. The chimera comprises CTLA-4 aa M1–I166 fused to CD28 aa L154–P218.

For generation of CD80mCD28 chimera, CTLA4mCD28 intracellular domains were amplified using 5'-CCAGAAGACCCTCCTGATTCAGACTTC CTCCTTTG-3' and 5'-ATATAAGATCTTCAGGGGCGGTACGCTG CAAA-3' and digested with *Bbv*11/*Bgl*11. The rCD80 extracellular domains were amplified using 5'-TTGAATTCCACCATGGCTTACAGTTGC CAGCTGA-3' and 5'-ATAGGATCCAGCAACGGAATTGT-3', digested with *Eco*R1/*Bbv*11, and ligated with the CTLA4mCD28 fragment into pczCFG5IZ *Eco*R1/*Bam*H1. The chimera comprises CD80 aa M1–P244 fused to CTLA-4 aa D159–I166 and CD28 aa L154–P218. Constructs in pczCFG5IZ/IEGN were transduced into 58 cells as described (7).

Stimulation, ELISA, and proliferation assays

For costimulation of cell lines (lanes 2 and 3 in Fig. 1, B and C, and Fig. 2B), plates for nonadherent cells (Greiner Bioscience) precoated with sheep anti-mouse IgG were coated with 2 μ g/ml TCR Ab, washed, and then coated with 10 μ g/ml protein G' (Sigma-Aldrich) to ensure immobilization of ligands containing human IgGfc. CD28 chimeras were ligated by the addition of 2 μ g/ml CD80/CD28/CTLA4Ig fusion proteins to 4×10^5 cells/ml in solution immediately before plating. For stimulation of CD28 chimeras alone (lane 4 in Fig. 1, B and C, and lanes 4 and 5 in Fig. 2B), plates were coated with mouse anti-human IgG, washed, and then coated with 10 μ g/ml protein G' (Sigma-Aldrich) to ensure high surface density of ligands. Cells were mixed with 10 μ g/ml soluble CD80/CD28/CTLA4Ig fusion proteins immediately before plating. IL-2 in the supernatant was assayed after 2–3 days using Opti-EIA kits (BD Biosciences).

For Fig. 4C, CD4⁺ T cells purified by nylon wool passage (1×10^6 /ml) were labeled with CFSE and stimulated with 10 μ g/ml E18 conventional or D665 superagonistic CD28 Abs on plates precoated with sheep anti-mouse IgG. After 4 days, CFSE dilution was measured by flow cytometry. Stimulation of purified T cells with all superagonistic mAbs require the addition of 5–10 μ g/ml mAb to cells in solution on plates, such as anti-mouse IgG-coated plates, that allow mAb immobilization at high density as described (6). For costimulation, CD28 mAbs can be added in solution at 10- to 100-fold lower concentrations to cells on TCR mAb-coated plates as described (13).

Generation of mouse anti-mCD28 Abs and surface plasmon resonance

CD28^{-/-} mice (B6.129S2-CD28^{tm1Mak/J} from The Jackson Laboratory) were immunized alternately with A20 cells expressing mCD28 i.p. and with recombinant mCD28Ig s.c. in TiterMax (Alexis). After boosting i.v. with CD28Ig, splenic cells were fused with X63Ag8.653 cells as described (7).

Abs were captured at 51–53 resonance units (RU) or 62–64 RU for D665 and E18, respectively, on an anti-mouse coated CM5 sensor surface (BR-1000-14; Biacore), and CD28Ig was repeatedly injected at concentrations of 1000 or 20 nM. Stoichiometry was calculated using the following equation: stoichiometry = $(RU_A/RU_{mAb}) \times (MW_{mAb}/MW_A)$, where MW is molecular weight. Binding of CD28 at 1000 nM to D665 and E18 was comparable, with stoichiometries of >1.5 CD28 homodimers per Ab, indicative of monovalent CD28 binding. Based on the assumption that both Abs bind with a stoichiometry of 2 at 1000 nM, stoichiometries were corrected assuming that 80 and 65% of D665 and E18 paratopes, respectively, are active.

Results

Bivalent ligation of CD28 with natural ligands induces IL-2 production in 58 TCR $\alpha\beta$ cells

To study the effect of CD28 ligation valency on costimulation, we first generated an experimental system that mimics physiological costimulation. We used 58 cells transduced with rat TCR, which produce only low levels of IL-2 after TCR ligation with R73 Ab (Fig. 1B) (7). 58 cells do not express detectable endogenous CD28, making this cell line appropriate for investigating the signaling capacity of ectopically expressed CD28 constructs. Stimulation of 58 TCR $\alpha\beta$ cells ectopically expressing wild-type CD28 with recombinant CD80Ig did not induce

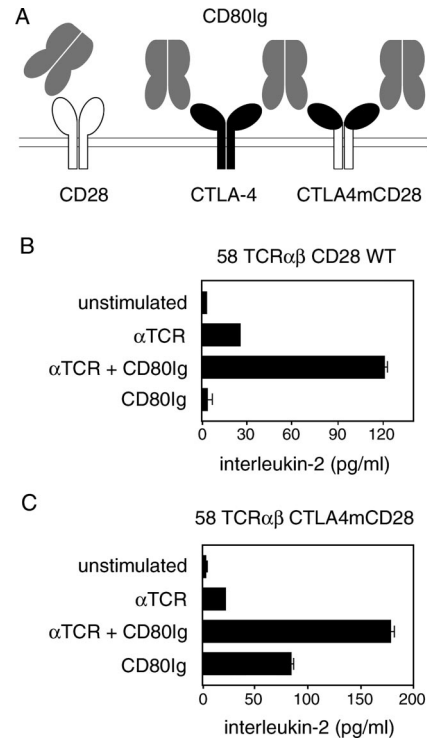


FIGURE 1. Bivalent ligation of CD28 with recombinant CD80Ig induces IL-2 production in 58 TCR $\alpha\beta$ cells. *A*, CD28 is monovalently ligated, whereas CTLA-4 is bivalently ligated by recombinant CD80Ig. The bivalent construct CTLA4mCD28 was generated by fusing the extracellular domains of CTLA-4 with the intracellular domains of CD28. *B* and *C*, 58 TCR $\alpha\beta$ cells expressing wild-type (WT) CD28 (*B*) or CTLA4mCD28 (*C*) were stimulated as indicated, and IL-2 production was measured by ELISA.

IL-2 production. However, costimulation via this receptor-ligand interaction induced high levels of IL-2 (Fig. 1B). Our system using 58 TCR $\alpha\beta$ cells expressing CD28 therefore corresponds to physiological costimulation with natural ligands in regard to the requirement for the ligation of both the TCR and CD28 in the induction of a functional response (6, 14).

Next, we generated a bivalent form of CD28. The extracellular domain of human CTLA-4 was fused with the transmembrane and intracellular domains of mCD28. The chimera, referred to as CTLA4mCD28, was expressed on 58 TCR $\alpha\beta$ cells at comparable levels to those of ectopically expressed wild-type CD28 on this cell line (data not shown). Stimulation of 58 TCR $\alpha\beta$ CTLA4mCD28 cells with TCR-specific Ab induced low levels of IL-2 production, which were markedly increased upon costimulation with CD80Ig (Fig. 1C). Importantly, stimulation of these cells with CD80Ig alone induced substantial IL-2 production. In this system, bivalent ligation alone is thus sufficient to induce cytokine production. Monovalent ligation of CD28 provides an efficient costimulatory stimulus but has no effect without TCR engagement.

A caveat of this interpretation is that we use an overexpression system to compare cell lines that may have differential sensitivity to costimulatory signals. To address these concerns, we designed an experimental system in which we directly compare differential ligation in the same cell line. By reversing the receptor-ligand pairs, we generated a CD28 chimera expressing the extracellular domains of CD80 fused to the intracellular domains of mCD28 (Fig. 2A). In contrast to Fig. 1, where we compare different CD28 constructs sharing the same ligand, we

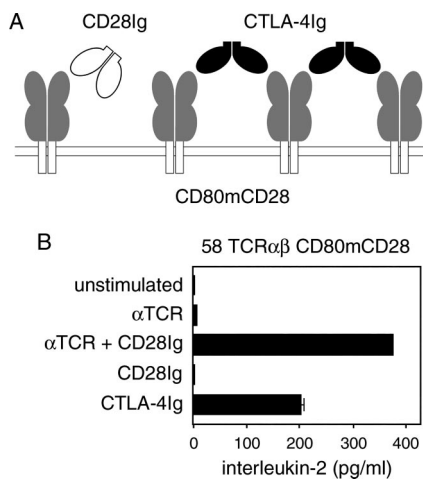


FIGURE 2. Ligation of CD28 with bivalent but not monovalent natural ligand pairs activates 58 TCR $\alpha\beta$ cells. *A*, A chimera comprising the extracellular domains of CD80 and intracellular domains of CD28 was stimulated with monovalent CD28Ig or bivalent CTLA-4Ig. IgGFc domains are not shown. *B*, 58 TCR $\alpha\beta$ cells expressing CD80mCD28 were stimulated as indicated, and IL-2 production was measured by ELISA.

now compare differential ligation of the CD80mCD28 construct by monovalent CD28Ig or bivalent CTLA4Ig ligands. Ligation with monovalent CD28Ig did not induce IL-2 production but provided an efficient costimulatory stimulus (Fig. 2*B*). By contrast, bivalent ligation with CTLA4Ig induced IL-2 production in the absence of TCR ligation.

Bivalent ligation of CD28 induces Ag-independent proliferative responses of primary peripheral T cells

To extend these findings to primary cells, we used novel Abs to mCD28 corresponding to previously described superagonistic and conventional Abs (6, 7, 13). Superagonistic Abs, such as 5.11A in human and JJ316 in rat, bind the laterally exposed C'D loop of CD28 and induce T cell proliferation without TCR ligation. Conventional Abs, such as JJ319 in rat and 37.51 in mouse, are strictly dependent on TCR ligation to induce T cell activation. For binding to CD28, both conventional Abs require integrity of residue 98 adjacent to the CD80 ligand binding loop and, thus, likely reflect natural ligand binding although with higher affinity. Two Abs (D665 and E18) that bound comparably well to mCD28 expressed on L cells (Fig. 3) were chosen. D665, hereafter referred to as a superagonistic Ab, induced proliferation of purified T cells, whereas E18, referred to as a conventional Ab, did not (data not shown, Fig. 4*C*). Binding of D665 to mCD28 grafted with the human C'D loop was severely diminished, whereas E18 binding was not affected. D665 therefore binds an epitope corresponding to that recognized by previously described CD28 superagonists (7). Binding of E18 to mCD28 grafted with the ligand binding region of rCD28 was abolished, but D665 binding was not affected. Given that the only difference between mouse and rat in the ligand binding region in the CD28 Ig domain is residue 98, E18 binds an epitope corresponding to that recognized by previously described conventional CD28 Abs.

We used surface plasmon resonance to assess the valency of CD28 for binding to D665 and E18 Abs. The two Abs were immobilized on a sensor surface at comparable levels and allowed to interact with recombinant CD28Ig homodimer in-

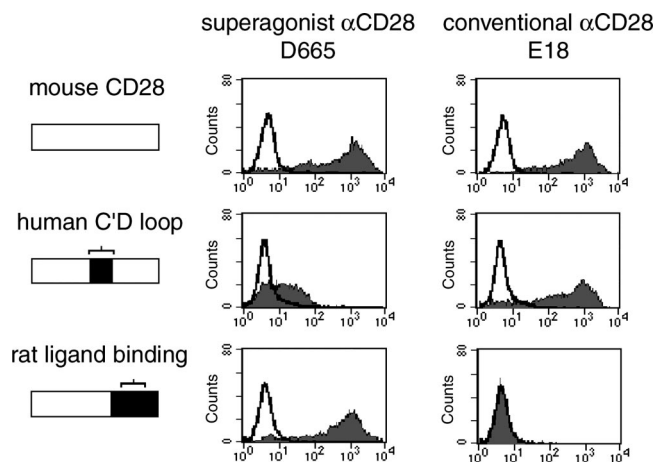


FIGURE 3. D665 Ab binds the C'D loop of mCD28, whereas E18 Ab binds to the ligand binding region. Binding of D665 (*middle panel*) and E18 (*right panel*) to L cells expressing CD28 constructs schematically depicted on the *left* is shown. Unshaded histograms show negative control staining.

jected on the surfaces at either high (Fig. 4*A*) or low (Fig. 4*B*) concentrations. At high concentrations, binding of CD28 to the two Abs was comparable with stoichiometries approaching 2, indicating that two CD28 homodimers are monovalently bound per Ab (Fig. 4*A*). Postinjection phases were similar, indicating similar dissociation rates and suggesting that the Abs have comparable affinity for CD28 (data not shown). At low concentrations, CD28 was bound by an E18 conventional Ab at a level approximately twice that of the D665 superagonist (Fig. 4*B*) with maximal stoichiometries of 1.6 and 0.7, respectively. These data indicate that under these conditions CD28 homodimers are predominantly ligated monovalently by the E18 conventional Ab but bivalently ligated by the D665 superagonist. The observation that the stoichiometry of the CD28/E18 Ab was <2 suggests that a proportion of CD28 may also be bivalently ligated by conventional Ab. The lower stoichiometry of 0.7 for CD28 complexed with the D665 superagonist, however, indicates that bivalent ligation of CD28 occurs more efficiently by the D665 superagonist than by the E18 conventional Ab.

To more rigorously test our earlier conclusion that superagonistic Abs induce T cell proliferation without the need for TCR ligation (6), we used C57BL/6 RAG^{-/-} mice transgenically expressing OT-2 TCR in which the TCR repertoire is restricted to one specificity (IA^b/Ova). Stimulation with the D665 superagonist induced proliferation of transgenic T cells, and no proliferation was induced by stimulation with the conventional Ab E18. Because neither the Ag nor the presenting MHC class II molecule was present in these cultures, this experiment provides an example of an artificial ligand that bivalently ligates CD28 and induces Ag-independent proliferation of primary T cells.

Discussion

The surprising finding that CD28 is monovalent whereas CTLA-4 is bivalent raises a variety of questions on the mechanisms of T cell costimulation. We show here that CD28 must be monovalent to induce a costimulatory responses without inducing responses in the absence of TCR engagement.

We use superagonistic Abs to demonstrate that, if appropriately ligated, CD28 has the potential to induce Ag-independent

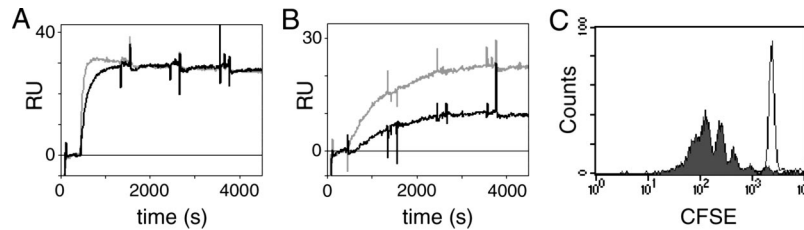


FIGURE 4. Superagonistic Ab D665, but not conventional Ab E18, bivalently ligates CD28 at low CD28/Ab ratios and induces Ag-independent proliferation of primary T cells. *A* and *B*, Recombinant CD28Ig at concentrations of 1000 nM (*A*) or 20 nM (*B*) was injected over a sensor surface with D665 (black line) or E18 (gray line) immobilized at comparable levels, and binding was measured over time. *C*, CFSE-labeled CD4⁺ T cells from C57BL/6 RAG^{-/-} OT-2 TCR transgenic mice were stimulated with D665 (shaded histogram) or E18 (open histogram), and proliferation was measured by CFSE dilution after 4 days.

T cell responses in primary peripheral T cells. We show in cell lines that such responses are also induced by bivalent ligation with natural ligands. By contrast, monovalent ligation with CD80Ig or the E18 conventional Ab induces efficient costimulatory responses without inducing responses in the absence of TCR ligation. We therefore conclude that monovalency of CD28 for its natural ligands is essential for its costimulatory function.

From the cocrystal structure of CD28 monomer and superagonistic 5.11A Fab', it is evident that this Ab bivalently ligates CD28, likely forming a CTLA-4-CD80 lattice-like structure (10). Surface plasmon resonance data indicate that mCD28 is also bivalently ligated by the superagonistic Ab D665 and predominantly monovalently ligated by the E18 conventional Ab (Fig. 4). We note that some other conventional Abs bivalently ligate CD28Ig fusion proteins (9, 10). But it is not clear whether this observation also holds true for binding of these Abs to cell surface-bound CD28. For example, the conventional human CD28-specific Ab 7.3B6 was shown by cryoelectromicroscopy to ligate CD28Ig fusion proteins at an angle that may well be incompatible with the structural constraints imposed on neighboring CD28 homodimers by their insertion into the cell membrane. Differences in the efficiency with which D665 superagonistic and E18 conventional Abs bivalently ligate CD28 may provide an explanation for the mechanistic difference between stimulation with these Abs.

Because superagonists bind to a membrane proximal epitope and parallel to the cell surface, whereas conventional Abs bind a membrane distal epitope, Evans et al. (10) argue that the different topology of ligated Abs explain their different stimulatory capacities. Closer membrane approximation to the immobilizing surface allowed by superagonists bound to CD28 may prevent access of large phosphatases like CD45, allowing signaling to proceed. By contrast, the extended structure of conventional Abs bound to CD28 may prevent close membrane approximation and allow access of phosphatases that limit signaling. Our data do not support this interpretation, because our ligated monovalent and bivalent recombinant ligands share the same dimensions. We favor an interpretation in which the periodicity and increased stability of CD28 complexes in a lattice formed by bivalent ligation (but not necessarily by all Abs that bivalently ligate CD28) allow efficient assembly of signaling complexes. The mechanisms of superagonist Ab stimulation remain, however, a moot point.

Another argument supporting the importance of monovalent CD28 ligation comes from the comparison of the stability of CD28 and CTLA-4 bound to CD80. Differential engagement by CD80 results in an ~100-fold difference in stability (9).

Given that CTLA-4 is expressed only at low levels on activated T cells or regulatory T cells, such differences in stability are likely to be essential for effective CTLA-4 function (15–17).

Lastly, early pharmacological studies suggested that the TCR/CD3 and CD28 signaling pathways are separate (18–20). However, a number of signaling molecules essential for TCR signaling, such as SLP-76 and Vav, are phosphorylated during superagonistic Ab stimulation (14). Moreover, although ligation of the TCR is not required for superagonistic signaling, expression of the TCR/CD3 complex is required (7). We therefore argue that CD28 is not an autonomous receptor. A likely explanation may be that CD28 signals amplify TCR signals downstream of TCR ζ , ZAP70, and LAT (linker for activation of T cells) at the level of the SLP-76 signalosome (14). Monovalent ligation of CD28 with either natural ligands or conventional Ab may provide a physiologically relevant amplification system, allowing costimulatory signaling without inducing responses in the absence of TCR ligation. In contrast, bivalent ligation may induce an amplification loop that amplifies the tonic TCR signals, which are required by T cells (21, 22), to a level that suffices to trigger functional T cell responses. In this scenario, bivalent CD28 ligation would induce a physiological but dysregulated signaling pathway. In this respect, superagonistic Abs will be an invaluable tool to dissect TCR and CD28 signal integration.

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Disclosures

T.H. declares a commercial interest in TeGenero Immunotherapeutics AG, Würzburg, Germany.

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